6706505



Figure 1A

((
S E C	SEQ O NO:	•		•	: .		
9	mouse_E3all	MASEMEPEVQ	AI D- RSLLEC	SAEEI AGRWL	QAT DL NREVY	QHL AHCVPKI	49
4	human_E3αl l	MASELEPEVQ	AID-RSLLEC	SAEEI AGKWL	QAT DL TREVY	QHL AHY VPKI	49
1.5	mouse_E3al	MADEEMDGAE	RIMDVSPEPPL	AP QR P AS WWD	QQVDFYTAFL	HHL AQL VPEI	50
7	human_E3al	MADEEAGGTE	MADEE AGGTE RIVE! SAEL PO	T P QR L AS WWD	QQVDFYT AFL	HHL AQL VPEI	50
	Cons ens us	MA. E	MA. E D L	W.	Q D	. HLA VP. I	50
٥	mouse_E3all	YCRGPNPFPQ	YCRGPNPFPQ KEDTLAQHIL	L GP ME WYI CA	EDPALGF PKL	EQANKPSHLC	66
4	human_E3al i	YCRGPNPFPQ	KEDML AQHVL	LGPMEWYLCG	EDPAFGFPKL	EQANKP SHLC.	66
15	mouse_E3al	YF AE MDP DL E	KQEESVQMSI	LTPLEWLFG	EDPDI CLEKL	KHSG- AFOLC	66
~	human_E3al	Y F AE MDP DL E	KQEESVQMSI	FTPLEWYLFG	EDPDI CLEKL	KHSG- AFQLC	66
	Consensus	γ	ж. 	L. P. EWYL. G. EDP KL	EDP KL	rc	100
						•	
9	mouse_E3aII	GRVFKVGEPT	YSCRDCAVDP	TCVLCNECFL	TCVLCMECFL GSI HRDHRYR	MITSGGGGFC	149
4	human_E3aII	GRVFKVGEPT	YSCRDCAVDP	TCVLCMECFL	GSI HRDHRYR	MITSGGGGFC	149
. 15	mouse_E3al	GKVFKSGETT	YSCRDCAI DP	TCVLCMDCFQ	SSVHKNHRYK	MHT STGGGFC.	149
7	human_E3al	GRVFKSGETT	YS CRDCAI DP	TCVLCMDCFQ	DSVHKNHRYK	MHT S T GGGF C	149
	Cons ens us	GRVFK. GE. T	YSCRDCA. DP	TCVLCM CF.	. S. H HRY.	M TS. GGGFC	150
				•			
9	mous e_E3aII	DCGDTEAWKE	GPYCOKHKLS	SSEVVEEEDP	L VHL SEDVI A	RTYNI FAI ME	199
4	human_E3aII	DCGDTEAWKE	GPYCOKHELN	TSELEEEDP	L VHL SEDVI A	RTYNI FAI TF	199
15	mous e_E3al	DCGDTEAWKT	GPFCVDHEPG	RAGTTKESLH	- CPLNEEVI A	QARRI FPSVI	198
7	human_E3al	DCGDTEAWKT	GPFCVNHEPG	RAGTI KENSR	- CPLNEEVI V	QARKI FPSVI	198
	Cons ens us	DCGDTE AWK.	GP. C. HE.		L . E. VI A	IF	200



Figure 1B

			•			•	
9	mouse_E3aII	RYAVDI LTWE	KESELPEDLE	VAEKSDTYYC	ML F NDE VHT Y	RYAVDI LTWE KESELPEDLE VAEKSDTYYC MLFNDEVHTY EQVI YTLOKA	249
4	human_E3aII	RYAVEI LTWE	KESELPADLE	KESELPADLE MVEKSDTYYC MLFNDEVHTY	ML F NDE VHT Ÿ	EQVI YTLQKA	249
15	mouse_E3al	KYI VEMTI WE	EEKELPPELQ	KYI VEMTI WE EEKEL PPELQ I REKNERYYC VLF NDEHHSY DHVI YSLORA	VL F NDE HHS Y	DHVI YS LORA	248
7	human_E3al	KYVVE MITI WE	EEKELPPELQ	KYVVEMTI WE EEKELPPELQ I REKNERYYC VLFNDEHHSY DHVI YSLQRA	VL F NDE HHS Y	DHVI YSLORA	248
	Consensus	. Y. VE WE	. E. ELP L.	. Y. VE WE . E. ELP L EK YYC . LFNDE. H. Y VI Y. LQ. A	. LFNDE. H. Y	. VI Y. LO. A	250
9	mous e_E3aII	VNCT QKE AI G	FATTVDRDGR	VNCT QKE A I G FATT V DR DGR RPVRYGDFQY CDQAKT VI VR NT SRQTK-PL	CDOAKTVI VR	NTSRQTK- PL	298
4	human_E3αII	VNCT QKE AI G	FATTVDRDGR	VNCTOKEAI G FATTVDRDGR RSVRYGDFOY CEQAKSVI VR NTSRQTK-PL	CEQAKS VI VR	NTSRQTK- PL	298
15	· mouse_E3al	LDCELAEAQL	HTTAI DKEGR	LDCELAEAQL HTTAI DKEGR RAVKAGVYAT CQEAKEDI KS HSENVSQHPL	CQE AKE DI KS	HS E NV S QHP L	298
7	human_E3¤l	LDCELAEAQL	HTTAI DKEGR	LDCELAEAQL HTTAI DKEGR RAVKAGAYAA CQEAKEDI KS HSENVSQHPL	CQEAKEDI KS	HSENVSQHPL	298
	Consensus	C EA	TDGR	T D GR R. V G C AK I PL	C AK I	P	300
9	mous e_E3aII	KVQVMHSSVA	AHONF GL KAL	S W GS VI GY S	DGL RRI LCQV	KVQVMHSSVA AHQNFGLKAL SWLGSVI GYS DGLRRI LCQV GLQEGPDGEN	348
4	human_E3aII	KVQVMHSSI V	KVQVMHSSI V AHQNFGLKLL	S W GS I I GYS	SW.GSIIGYS DGLRRILCQV GLOEGPDGEN	GL QE GP DGE N	348
. 15	mous e_E3al	HVEVLHSVVM	AHQKF AL RL G	HVEVLHSVVM AHQKFALRLG SWMNKI MSYS SDFRQI FCQA CLVEEPGSEN	SDFRQI FCQA	CLVEEPGSEN	348
7	human_E3al	HVEVLHSEI M	AHOKF AL RL G	S WANK! NG YS	S DF RQI FCQA	HVEVLHEEI M AHOKFALRLG SWANKI MSYS SDFROI FCOA CLREEPDSEN	348
	Cons ens us	. V. V. HS	AHO. F. L. L.	SW I YS	R. I . CQ.	. V. V. HS AHO. F. L. L. SW I YS R. I . CQ L. E. P.D. EN	350



Figure 1C

398 398 398 398	448 448 448 450
ALRFAKNYRQ AVRFAKNYQQ AMEFVKYYKQ AMEFVKYYKQ	TVI I KAFMDH SI I I KTFMDH SVI TETLLEV SVI TETLLEV SVI . T
LWDLKYKKLF LWDLKYKKLF FWENEYKKLF FWENEYKKLF .MYKKLF	RMLLTEENLM RMLITEENLM RHLIEEONVI RHLIEEONVI
VYHQLFMSSL VYHQLFMSSL I LHELI FSSF I LHELI FSSF H. LSS.	VQFFTAPTLA VQFFTAPTLA VQMLTVPTLA VQMFTVPTLA
SSLVDRLMLN DSKLWKGARS VYHQLFMSSL LMDLKYKKLF ALRFAKNYRQ SSLVDRLMLS DSKLWKGARS VYHQLFMSSL LMDLKYKKLF AVRFAKNYQQ PCLISRLMLW DAKLYKGARK I LHELIFSSF FMEMEYKKLF AMEFVKYYKQ PCLISRLMLW DAKLYKGARK I LHELIFSSF FMEMEYKKLF AMEFVKYYKQLRLML. D. KL. KGAR H. L SS M YKKLF A. F. K. Y. Q	LQRDF.MEDDH ERAVSVTALS VQFFTAPTLA RMLLTEENLM TVI I KAFMDH LQRDF.MEDDH ERAVSVTALS VQFFTAPTLA RMLITEENLM SI I I KTFMDH LQKEYI SDDH ERSI SI TALS VQMLTVPTLA RHLI EEQNVI SVI TETLLEV LQKEYI SDDH DRSI SI TALS VQMFTVPTLA RHLI EEQNVI SVI TETLLEV LQKEYI SDDH DRSI SI TALS VQMFTVPTLA RHLI EEQNVI SVI TETLLEV LQ DDH ER S. TALS VQ. FT. PTLA R. LI . E. N SVI T
mous e_E3all huma n_E3all mous e_E3al huma n_E3al Cons ens us	mous e_E3aII human_E3aII mous e_E3aI human_E3aI
6 15 2	6 1 2 2 2



Figure 1D

	I LDLKYVLI SK PTEWS DELRO 498	I LDLKYVLI SK PTEWS DELRO 498	1 CDLKYI LI SK PVI WTERLRA 496		1 . DLKY. LI SK PT. W LR. 500	II EMEPEWEAAF TLOMKLTHVI 548	HI EMEPEWEAAF TLOMKLTHVI 548	II EVDP.DWEAAI AI QMQLKNI L 546	HI EVDPDWEAAI AI QMQLKNI L 546	11 E. P. WE AA QM L 550	SINVODWCALD EKYLI EAYKK CLAVLTQCHG GFTDGEQPIT LSI CGHSVET 598	CLAVLMQCHG GYTDGEQPIT LSI CGHSVET 598		T NFMSTKTV- VOLCGHSLET 595
	QFERYTAL QA FKFRRVQSLI	OFERYTALOA FKFRRVOSLI	KEN-FQGYSQ DKLGRVYAVI	KFN- FQGYSQ DKLGRVYAVI	. F K RV 1	ELLIKCMOGMO PI TROVGOHI	ELLKCMOGND PI TROVGOHI	KILTCMOGNE EI RRQVGQHI	CILTCMOGNE EI RROVGOHI	. L. CMDGM . I . RQVGQHI	KVLI EAYKK CLAVLTOCH			L MF QE WCACD EDLLL VAYKE CHKAV MRCST NF MS STKTV-
.•	LKHRDAQGRF	LRHRDAGGRF	LPEYLDRNN- K	L PEYL DRNN- K	L	KFLQGFDAFL E	KFLEGFDAFL E	QFLEGFRSFL K	OFLEGFRSFL KILTCMOGNE	. FLEGF FL L. CMDGM	S MY Q DWCALD E	S MWQDWCASD EKVLI EAYKK		LIMF QE WCACD E
SEQ ID NO:	mouse_E3aII	human_E3aII	mouse_E3al	human_E3¤l	Consensus.	mous e_E3aII	human_E3aII	mouse_E3αl	human_E3al	Cons ens us	mous e_E3aII	human_E3αII		mous e_E3&I
SEQ	9	4	15	7		9	4	15	7		9	4	5	1



Figure 1E

9 3 1 0	E3.011	I BY CVS OF KV	I as id in is	SAS FINH ISA	BYCVSOEKV SI HI PI SBI I AGI HVI SKS EVAVKEDELI DI SEI SDOM	DICEICODAM	
human_E3aII I YCVS	I YCVS	GEKV S	SI HLPVSRLL	AGL HVL L S KS	I YCVS OEKV SI HLPVSRLL AGLHVLLSKS EVAYKFPELL PLSELSPPM	PLSELSPPML	648
	KSYKV	SEDLV	SI HLPLSRTL	AGL HVRL SRL	KSYKVSEDLV SI HLPLSRTL AGLHVRLSRL GAI SRLHEFV PFDSFQVEVL	PFDSFQVEVL	645
human_E3al KSYRV	KSYRV	SEDLV.	SIHLPLSRTL	AGL HVRL SRL	GAVSRLHEFV	SFEDFQVEVL	645
ConsensusY.V	> .	SV	SI HLP. SR. L	AGLHV. LS	Y. VS V SI HLP. SR. L AGLHV. LS E P L	P L	650
mouse_E3aII I EHPL	I EHPL	I EHPLRCLVL	CAQVHAGMMR RNGFSLVNQI	RNGFSLVNQI	YYYHNVKCRR	YYYHNVKCRR EMFDKDI VML	698
human_E3aII I EHPLRCLVL	I EHPL	RCLVL	CAQVHAGMMR	CAQVHAGMMR RNGFSLVNQI	YYYHNVKCRR	YYYHNVKCRR EMFDKDVVML	698
mouse_E3al VEYPLRCLVL	VEYPLA	ICL VL	VAQVVAEMMR	VAQVVAEMMR RNGLSLI SQV	FYY QDVKCRE EMY DKDI I ML	EMYDKDI I ML	695
human_E3al VEYPLRCLVL	VEYPL	RCLVL	VAQVVAEMMR	RNGL SLI SQV	VAQVVAEMMR RNGLSLISQV FYYQDVKCRE EMYDKDIIM	E MY DKDI I ML	695
Consensus . E. PL	. E. PL	. E. PLRCLVL		. AQV. A. MMR RNG. SL Q YY VKCR.	. YY VKCR.	EM DKDI . ML	700
mouse_E3aII QTGVS	QTGVS	MWDP N	HFLM MLSRF	ELYQLFSTPD	QTGVSMMDPN HFLM MLSRF ELYQLFSTPD YGKRFSSEVT HKDVVQQNNT	HKDVVQQNNT	. 748
	QTGVS	MWDP N	HFLM MLSRF	ELYQI FSTPD	CTGVS MMDPN HFLM MLSRF ELYQI FSTPD YGKRFSSEIT	HKDVVQQNNT	748
mouse_E3al QIGASI	QI GASI	MDPN	QI GASI MDPN KFLLLYLQRY	ELTDA	EL TDA FNKT! STK DQDLI KQYNT	DODLI KOYNT	738
human_E3al QI GAS	QI GAS	L MDP N	KFLLLVLORY	EL AEA	QI GASL MDPN KFLLL VLQRY EL AEA FNKTI ST K DQDLI KQYNT	DODL! KQYNT	738
Consensus Q. G. S	9.6.5	MDPN.	. FL L. R.	ELT.	Q. G. S. MDPN . FL L. R. EL T K S D Q. NT	D. O. NT	750



Figure 1F

				1			
9	mouse_E3aII		LI EEMLYLII MLVGERFNPG VGQVAATDEI	3 VGQVAAT DEI	KREI I HQLSI	KP MAHS EL VK	798
4	human_E3αII	LI EEMLYLI I	MLVGERFSPG	3 VGQVNAT DEI	KREI I HOLSI	KP MAHS EL VK	798
15	mouse_E3al	LI EEMLQVLI	YI VGERYVPG	YI VGERYVPG VGNVTREEVI	MREI THLLCI	EPMPHSAI AR	788
7	human_E3al	LI EEMLOVLI		YI VGERYVPG VGNVTKEEVT	T MREI I HLLCI		.788
	Consensus	LI EEMLI		VGER PG VG. VI	. REI I H. L. I	. P.M. HS K	80(
9 .	mouse_E3aII		GMESVIESVA	N HFKKPGLTG	R GMYELKPEC	SLPEDENKET GMESVIESVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF	848
4	human_E3αII	SLPEDENKET	SLPEDENKET GMESVI EAVA HFKKPGLTGR	N HFKKPGLTG	R GMYELKPEC	GMYELKPECA KEFNLYFYHF	848
15	mouse_E3al	NL PENENNET	NLPENENNET GLENVI NKVA TFKKPGVSGH	TFKKPGVSG		KDFNMYFYHY	838
7	human_E3al	NL P E NE NNE 1	GLENVI NKV	A TFKKPGVSGI	NLPENENNET GLENVI NKVA TFKKPGVSGH GVYELKDESL	. KDF NMYFYHY	838
	Consensus	. LPE. EN. ET	. LPE. EN. ET G. E. VI VA . FKKPG G.	A . FKKPG. G.	G. YELK. E.	K. FN. YFYH.	85.
9	mous e_E3aII	SRAEQSKAEE	AQRKLKRENK	AQRKLKRENK EDTALPPPAL	PPFCPLFASL	VNI LOCDVM	868
4	human_E3αII	SRAEQSKAEE	AQRKL KRQNR	EDTALPPPVL	PPFCPLFASL	VNI LQS DVML	89
15	mouse_E3al	SKTQHSKAEH	MQKKRRKQEN KDEALPPPPP PEFCPAFSKV	KDEALPPPP	PEFCPAFSKV	VNL L SCDVM	88
7	human_E3αl	SKTQHSKAEH	SKTQHSKAEH MQKKRRKQEN KDEALPPPPP PEFCPAFSKV	KDEALPPPP	PEFCPAFSKV	I NL L NCDI MM	88
	Cons ens us	S SKAE.	. O. K O.	. D. ALPPP	S SKAE Q. K Q D. ALPPP P. FCP. F	VN. L. CDVM	90



Figure 1G

948 948 937	937	998 998	987	1000	046	037	034
GMALQEEKHH LENAVEGHVQ GMALQEEKQH LENVTEEHVV ALGLLEEKQQ LQKAPEEEV-	L QKAPEEEV- L A. EE. V.	I RWLLKWFNA I RW LKTFNA	I TW LOWEDT I TW LOWEDT		I ARLRREKI M 1046 I ARLRREKI M 1046	AARLHRQKI M 1037	AARLHRQKI M 1034 . ARL. R. KI M 1050
GMALQEEKHH LENAVEGHVQ GMALQEEKQH LENVTEEHVV ALGLLEEKQQ LQKAPEEEV-	ALGLLEEKQQ LQKAPEEEV- L.EEKQ. LA.EE.V.	TFTFTQKISK PGDAPHNSPS ILAMLETLON APSLEAHKDM IRWLLKMFNA TFTFTQKISK PGEAPKNSPS ILAMLETLON APYLEVHKDM IRW LKTFNA	AFDFYHKASR LGSSAMNAQN I QMLLERLKG I PQLEGQKDM I TW LQMFDT TFDFYHKASR LGSSAMNI QM LLEKLKG I PQLEGQKDM I TW LQMFDT	. P. LE KDM	DKAERKRKAE DKAERKRKAE	EKAERKRKAE	EKAERKRKAE . KAERKRKAE
SMLQRVLHLI SMLQRVLHLI GMLQMAFHIL	YI LRTVFERA I DTDS NLWTE GMLQMAFHI LYITIA VW.E.MLQH	I LAMLETLON I LAMLETLON	I OMLLERLKG LLEKLKG	I LE. L	IKKIRECS SSSPVAEAEG TIMEESSRDK VKKMRESS PTSPVAETEG TIMEESSRDK	VKRLREKSCL VVATTSGLEC I KSEE! THDK EKAERKRKAE	VKRLREKSCL I VATTSGSES I KNDEI THDK EKAERKRKAE VK RE C EE DK . KAERKRKAE
YIMGTILQWA VEHHGSAWSE SMLQRVLHLI CIMGTILQWA VEHNGYAWSE SMLQRVLHLI YILRTIFERA VDTESNLWTE GMLQMAFHIL	YILRTVFERA I DTDS NLWTE GMLQMAFHILYITIA VW.E.MLQH	PGDAPHNSPS PGEAPKNSPS	LGSSAMNAQN LGSSAMNI QM		SSSPVAEAEG PTSPVAETEG	VVATTSGLEC	I VATTSGSES
YI MGTI LQWA CI MGTI LQWA YI LRTI FERA	YI LRTVFERA YI TI A	TFTFTQKI SK TFTFTQKI SK	AF DF Y HKAS R T F DF Y HKAS R	TF. F K. S.	I KKI RE CS VKKMRE SS	VKRLREKSCL	VKRLREKSCL VKREC.
SEQ I D NO: 6 mous e_E3αII 4 huma n_E3αII 15 mous e_E3αI	human_E3œl Consensus	mouse_E3all human_E3all	mouse_E3α human_E3α	Cons ens us	mouse_E3αII human_E3aI	mouse_E3at	huma'n_E3αl Consensus
SEQ 6 . 4 . 15	7	o 4	15		ο 4	15	7



Figure 1H

1094 1094 1087 1084 1100	1144 11137 1134 1150	1193 1193 1184 1181
AQMSEMORHF I DENKELFQQ TLELDTSASA TLDSSPPV SDAALTALGP 1094 AQMSEMORHF I DENKELFQQ TLELDASTSA VLDHSPVA SDMTLTALGP 1094 AQMSALQKNF I ETHKLMYDN TSEVTGKEDS I MEEESTSAV SEASRI ALGP 1087 AQMSALQKNF I ETHKLMYDN TSEMPGKEDS I MEEESTPAV SDYSRI ALGP 1084 AQMS. Q. F I K T. E S. P. V SD ALGP 1100	VLSKDRTKTI VLSKNRSKFI ALTQHRGKPV ALTQHRGKPI	AD-PEKYDPL FMHPDLSCGT HTGSCGHVMH AHCWQRYFDS VQAKEQRRQQ 1193 QD-PEKYDPL FMHPDLSCGT HTSSCGHI MH AHCWQRYFDS VQAKEQRRQQ 1193 DHLGETLDPL FMDPDLAHGT YTGSCGHVMH AVCWQKYFEA VQLSSQQ 1181 ELSGEALDPL FMDPDLAYGT YTGSCGHVMH AVCWQKYFEA VQLSSQQ 1181 CLSGEALDPL FMDPDLAYGT YTGSCGHVMH AVCWQKYFEA VQLSSQQ 1181 CLSGEALDPL FM PDL. GT . TGSGGHVMH A.CWQ.YF. VQ QQ 1200
TLDSSPPV VLDHSPVA I MEEESTSAV I MEEESTPAV	VLAAFVQRST VLAAFVQRST VLSACVQKST VLSACVQKST VLSACVQKST	AHCWQRYFDS AHCWQRYFDS AVCWQKYFEA AVCWQKYFEA
TLELDTSASA TLELDASTSA TSEVTGKEDS TSEMPGKEDS T.E	QEVTVGSRAM QEVKVESRAM QEVKLENNAM QEVK!ENNAM	HT GS CGHVMH HT S S CGHI MH YT GS CGHVMH YT GS CGHVMH
I DENKELFQQ I DENKELFQQ I ETHKLMYDN I ETHKLMYDN I ETHKLMYDN	AQTQVPEPRQ FVTCI LCQEE QEVTVGSRAM VLAAFVQRST VLSKDRTKTI TQTQVPEQRQ FVTCI LCQEE QEVKVESRAM VLAAFVQRST VLSKNRSKFI KRGPAVTEKE VLTCI LCQEE QEVKLENNAM VLSACVQKST ALTQHRGKPV KRGPSVTEKE VLTCI LCQEE QEVKI ENNAM VLSACVQKST ALTQHRGKPI	FWHP DL SCGT FWHP DL SCGT FWDP DL AHGT FWDP DL AYGT FW PDLGT
AQMS E MQRHF AQMS E MQRHF AQMS AL QKNF AQMS AL QKNF AQMS Q F	AQTQVPEPRQ TQTQVPEQRQ KRGPAVTEKE KRGPSVTEKE	AD- PEKYDPL QD- PEKYDPL DHLGETLDPL ELSGEALDPL
mouse_E3αII human_E3αII mouse_E3αI human_E3αI Consensus	mouse_E3αII human_E3αII mouse_E3αI human_E3α Consensus	mous e_E3aII huma n_E3aII mous e_E3aI huma n_E3aI Cons ens us
6 1 5 2	6 4. t 2 5 5	6 2 2 2



Figure 11

1241 1241 1233 1230 1250.	1290 1290 1283 1279 1300	1340 1340 1333 1329
L-LPPRSILS RRLN-FSDQP L-LPPRNIFN NRLN-FSDQP IPLQPQKINS ENAEALAQLL IPLQPQKINS ENADALAQLL 	SSEDTEAMNI IPIPEGFRPD 1290 STKNSENVDE LQLPEGFRPD 1290 LFNQGMGDST FEFHSILSFG 1283 FFNQGMGDST LEFHSILSFG 1279	L CWGT CAYT! MCWGS CAYT! MT WS T CAFT! L T WS T CAFT!
L-LPPRSILS RRLN-FSDQP 1241 L-LPPRNIFN NRLN-FSDQP 1241 IPLQPQKINS ENAEALAQLL 1233 IPLQPQKINS ENADALAQLL 1230 1250	QQI KVVQMLR RKHNAA-DTS SSEDTEAMNI I PI PEGFRPD QQI KALQFLR KEESTP-NNA STKNSENVDE LQLPEGFRPD ARI SGYNI KH AKGEAPAVPV LFNQGMGDST FEFHSI LSFG ARI SGYNI RH AKGENP-I PI FFNQGMGDST LEFHSI LSFG	FYPRNPYSDS I KEMLTTFGT AAYKVGLKVH PNEGDPRVPI LCWGTCAYTI FRPKI PYSES I KEMLTTFGT ATYKVGLKVH PNEEDPRVPI MCWGSCAYTI VQSSVKYSNS I KEMVI LFAT TI YRI GLKVP PDELDPRVPM MTWSTCAFTI VESSI KYSNS I KEMVI LFAT TI YRI GLKVP PDERDPRVPM LTWSTCAFTI YS. S I KEM. F.T. Y. GLKV. P.E. DPRVP. W.TCA.TI
RLRLHTSYDV ENGEFLCPLC ECLSNTVI PL RLRLHTSYDV ENGEFLCPLC ECLSNTVI PL RI HVDL-FDL ESGEYLCPLC KSLCNTVI PI RI HVDL-FDL ESGEYLCPLC KSLCNTVI PI R D. E. GE. LCPLC L. NTVI P.	DLAQWTRAVT QQI KVVQMLR RKHNAA- DTS SSEDTEAMNI NLTQM RTI S QQI KALQFLR KEESTP- NNA STKNSENVDE TLARW QTVL ARI SGYNI KH AKGEAPAVPV LFNQGMGDST TLARW QTVL ARI SGYNI RH AKGENP- I PI FFNQGMGDST LA. W. TV	FYPRNPYSDS I KEMLTTFGT AAYKVGLKVH PNEGDPRVPI FRPKI PYSES I KEMLTTFGT ATYKVGLKVH PNEEDPRVPI VQSSVKYSNS I KEMVI LFAT TI YRI GLKVP PDELDPRVPM VESSI KYSNS I KEMVI LFAT TI YRI GLKVP PDERDPRVPN YS. SIKEMI LFAT TI YRI GLKVP PDERDPRVPN
ENGEFICPLC ECLSNTVI PL ENGEFICPLC ECLSNTVI PL ESGEYLCPLC KSLCNTVI PI ESGEYLCPLC KSLCNTVI PI E. GE. LCPLCL. NTVI P.	QQI KVVQMLR RKHNAA- DTS QQI KALQFLR KEESTP- NNA ARI SGYNI KH AKGEAPAVPV ARI SGYNI RH AKGENP- I PI	FYPRNPYSDS I KEMLTTFGT AAYKVGLKVH FRPKI PYSES I KEMLTTFGT ATYKVGLKVH VQSSVKYSNS I KEMVI LFAT TI YRI GLKVP VESSI KYSNS I KEMVI LFAT TI YRI GLKVP YS. S I KEM
RLRLHTSYDV RLRLHTSYDV RI HVDL-FDL RI HVDL-FDL R D.	DLAQWTRAVT NLTQM RTI S TLARW QTVL TLARW QTVL	FYPRNPYSDS FRPKI PYSES VQSSVKYSNS VESSI KYSNS
mbuse_E3aII human_E3aII mbuse_E3aI human_E3aI Consensus	mouse_E3aII human_E3aII mouse_E3aI human_E3aI	mouse_E3aII human_E3aII mouse_E3aI human_E3aI
6 1 1 2	6 4 4 2 2 2	6 4 1 2



Figure 1J

SEQ ID NO:						
6 mouse_E3aII	QSIERILSDE	EKPVFGPLPC	RLDDCLRSLT	RFAAAHWTVA	LLPVVQGHFC	1390
4 human_E3aII	QSIERILSDE	DKPLFGPLPC	RLDDCLRSLT	RFAAAHWTVA	SVSVVQGHFC	1390
15 mouse_E3aI	QAIENLLGDE	GKPLFGALQN	RQHSGLKALM	QFAVAQRATC	PQVLIHKHLA	1383
2 human_E3αI	QAIENLLGDE	GKPLFGALQN	RQHNGLKALM	QFAVAQRITC	PQVLIQKHLV	1379
Consensus	Q . IE L .DE	Q. JE. L.DE . KPLFG. L	RLL.	. FA . A	Q.H.	1400
6 mouse_E3aII	KLFASLVPSD	SYEDLPCILD	IDMFHLLVGL	VLAFPALQCQ	DFSGSSL	1437
4 human_E3aII	KLFASLVPND	SHEELPCILD	IDMFHLLVGL	VLAFPALQCQ	DFSGISL	1437
15 mouse_E3aI	RLLSVILPNL	QSENTPGLLS	VDLFHVLVGA	VLAFPSLYWD	DTVDLQPSPL 1433	1433
2 human_E3αI	RLLSVVLPNI	KSEDTPCLLS	IDLFHVLVGA	VLAFPSLYWD	DPVDLQPSSV 1429	1429
Consensus	. L PN.	E PC .L .	ID. FH. LVG.	VLAFP. L	D SSL	1450
d to						
mouse_E3all	ATGDLHIF	HLVTMAHIVQ	ILLTSCTEEN	GMDQENP	TGEEELAILS	1482
4 human_E3aII	GTGDLHIF	HLVTMAHIIQ	ILLTSCTEEN	GMDQENP	PCEEESAVLA	1482
15 mouse_E3al	SSSYNHLYLF	HLITMAHMLQ	ILLTTDTDLS	PGPPLAEGEE	DSEEARCASA	1483
2 human_E3αI	SSSYNHLYLF	HLITMAHMLQ	ILLTVDTGL-	PLAQVQE	DSEEAHSASS	1475
Consensus	F	HL. TMAH Q	ILLTT	0	. EE	1500



Figure 1K

532 532 533 525 525	581 581 583 575 600	631 631 633 625 650
FHYLNGVPAP 1532 FHYLNGVPSP 1532 FHYLLGVAPP 1533 FHYLLGVTPP 1525 FHYL.GV.P 1550	LPTNLIHLFQ ENSDIMNSLI ESWCQNSEVK 1581 LPNNLICLFQ ENSEIMNSLI ESWCRNSEVK 1581 LPTNLFLLFQ EYWDTIRPLL QRWCGDPALL 1583 LPTNLFLLFQ EYWDTVRPLL QRWCADPALL 1575 LPTNLFLLFQ EDLWC 1600	NQASNFSCPK SGGDKSRAPT 1631 NQASNFSCPK SGGDKSRAPT 1631 NQASHFRCPR SADDERKHPV 1633 NQASHFRCPR SADDERKHPV 1625 NQAS.F.CP. SDP. 1650
MPFLKCSAL MPFLKCSALF TPYLRCAALL TPYLRCAALF	LPTNĻIHLFQ ENSDIMNSLI LPNNLICLFQ ENSEIMNSLI LPTNLFLLFQ EYWDTIRPLL LPTNLFLLFQ EYWDTVRPLL LPTNLLFQ EDL.	NQASNFSCPK NQASNFSCPK NQASHFRCPR NQASHFRCPR NQAS · F · CP.
SALKEAPSGW HLWRSVRAAI MPFLKCSAL SALKEIPSGW HLWRSVRAGI MPFLKCSALF GLTGCGAPGW YLWLSLRNGI TPYLRCAALL GSIGCDIPGW YLWVSLKNGI TPYLRCAALF	LPTNLIHLFQ ENSDIMNSLI LPNNLICLFQ ENSEIMNSLI LPTNLFLLFQ EYWDTIRPLI LPTNLFLLFQ EYWDTVRPL	DLPEDYSSLI NLPEDYSSLI ELPEDYSCLL ELPDDYSCLL
SALKEAPSGW SALKEIPSGW GLTGCGAPGW GSIGCDIPGW	HFEHLCNYLS HFEHLCSYLS EFSALCSYLS EYSALCSYLS . F LCSYLS	SYPRGANKLI RYPRESNKLI RYPRKRNSLI RYPRKRNSLI RYPR N. LI
LHKTLHQYTG LYKTLHQYTG FFVEVSQHTD FFAEISQYTSQYT.	PDLQV-SGTS PDIQV-PGTS EELFANSAEG EELHTNSAEG	RYLNGERGAI RYLEGERDAI KSLKOKSAVV NCLKOKNTVV
6 mouse_E3αII 4 human_E3αII 15 mouse_E3αI 2 human_E3αI Consensus	6 mouse_E3αII 4 human_E3αII 15 mouse_E3αI 2 human_E3αI Consensus	6 mouse_E3all 4 human_E3all 15 mouse_E3al 2 human_E3al Consensus



Figure 1L

1681 1681 1683 1675 1700	1731 1731 1733 1725 1725	
I FLRVRECQV I FLRVRECQV I FLKI RECRV I FLKI RECRV I FL REC. V	RKI QKLWQQH KKI QKLWHQH RKLHLVWQQH RKLHLVWQQH RKWQQH	· · · · · · · · · · · · · · · · · · ·
EGEDVGACTA HTYSCGSGAG IFLRVRECQV 1681 EGEDVGACTA HTYSCGSGVG IFLRVRECQV 1681 NGEEVGACVF HALHCGAGVC IFLKIRECRV 1683 NGEEVGACIF HALHCGAGVC IFLKIRECRV 1675	NPL HL CQERF NPL HL CKERF NPL HL SRERY NPL HL SRERY NPL HL SRERY	1755 1757 1749 1774
EGEDVGACTA EGEDVGACTA NGEEVGACVF NGEEVGACIF	GETDQGL RRG GETDQGL RRG GETDPGL KRG GETDPGL KRG GETD. GL. RG	
AEL TEL EL V	Ο Ε Υ Ε Υ	
SQS YCCQ SQS YCCQ SQNI CCQE SQNI CCQE SQNI CCQE	FYSPPYLD FYSPPYLD AYPAPYLD AYPAPYLD . Y PYLD	EANQTLVG EANQTLVG ETNQMLFGF ETNQMLFGF
LCLVCGSLLC SQSYCCQAEL EGEDVGACTA HTYSCGSGAG IFLRVRECQV 1681 LCLVCGSLLC SQSYCCQTEL EGEDVGACTA HTYSCGSGVG IFLRVRECQV 1681 LCLFCGAILC SQNI CCQEIV NGEEVGACVF HALHCGAGVC IFLKI RECRV 1683 LCLFCGAILC SQNI CCQEIV NGEEVGACIF HALHCGAGVC IFLKI RECRV 1675 LCLFCGAILC SQNI CCQEIV NGEEVGACIF HALHCGAGVC IFLKI RECRV 1675	LFLAGKTKGC FYSPPYLDDY GETDQGLRRG NPLHLCQERF RKI QKLWQQH 1731 LFLAGKTKGC FYSPPYLDDY GETDQGLRRG NPLHLCKERF KKI QKLWHQH 1731 VLVEGKARGC AYPAPYLDEY GETDPGLKRG NPLHLSRERY RKLHLVWQQH 1733 VLVEGKARGC AYPAPYLDEY GETDPGLKRG NPLHLSRERY RKLHLVWQQH 1725 GK GCYPYLD.Y GETD.GL.RG NPLHL.ER. RKWQQH 1750	SITEEI GHAQ EANQTLVGI D WQHL SVTEEI GHAQ EANQTLVGI D WQHL CI I EEI ARSQ ETNQMLFGFN WQLL CI I EEI ARSQ ETNQMLFGFN WQLL
mouse_E3aII LCLVCGSLLC SQSYCCQhuman_E3aII LCLVCGSLLC SQSYCCQmouse_E3ai LCLFCGAILC SQNI CCQEhuman_E3ai LCLFCGAILC SQNI CCQIConsensus LCL.CGLC SQCCQ	mouse_E3aII LFLAGKTKGC FYSPPYLD human_E3aII LFLAGKTKGC FYSPPYLD mouse_E3aI VLVEGKARGC AYPAPYLD human_E3aI VLVEGKARGC AYPAPYLD ConsensusGKGC .YPYLD	mouse_E3\alphaII SITEEIGHAQ EANQTLVGID WQHL human_E3\alphaII SVTEEIGHAQ EANQTLVGID WQHL mouse_E3\alphaI CIIEEIARSQ ETNQMLFGFN WQLL human_E3\alphaI CIIEEIARSQ ETNQMLFGFN WQLL Consensus I.EEIQ E.NQ.L.G. WQ.L



FIG. 2

Tth Expression Profile of huE3 α -II in Human Tissues

Brain
Heart
Skeletal muscle
Colon
Thymus
Spleen
Kidney
Liver
Small intestine
Placenta
Lung
Leukocyte

9.5kb —

7.5kb —

4.4kb —

2.4kb ---



FIG. 3

Tth Expression Profile of huE3 α -l in Human Tissues

Heart Brain Placenta Lung Liver Skeletal Musck Kidney

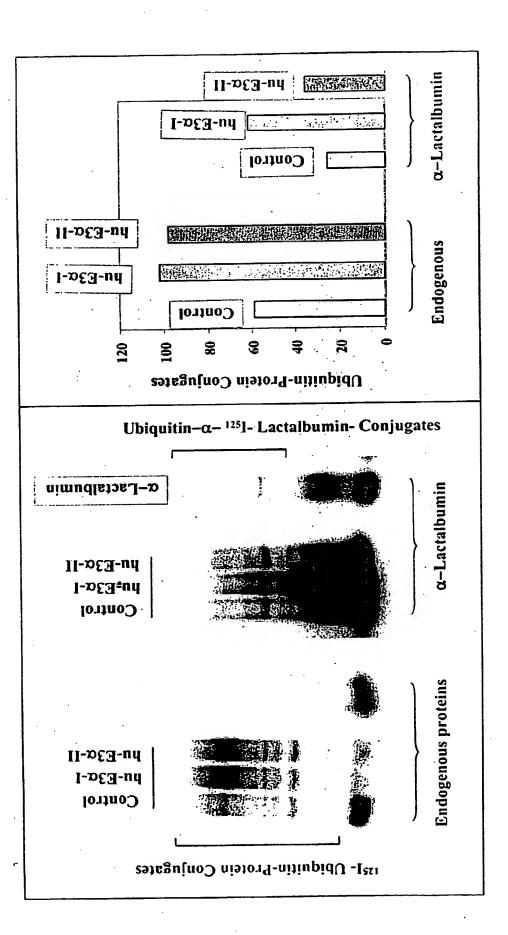
9.5kb __ 7.5kb __

4.4kb —

2.4kb —

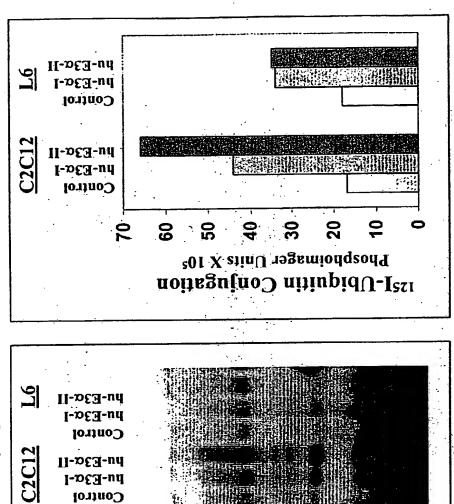


Figure 4
Ubiquitination of Endogenous Proteins



Transfection of Human E3a-I or E3a-II cDNA Stimulates Ubiquitin Conjugation in Cultured Muscle Cell Lines

hu-E3a-II pn-E3\a-1 Control



Conjugates

125I-Ubiquitin-Protein



Figure 6

 $^{125}\text{I-Ubiquitin}$ Conjugation to Muscle Proteins and Its Sensitivity to E3 α Inhibitor in Skeletal Muscle Extracts

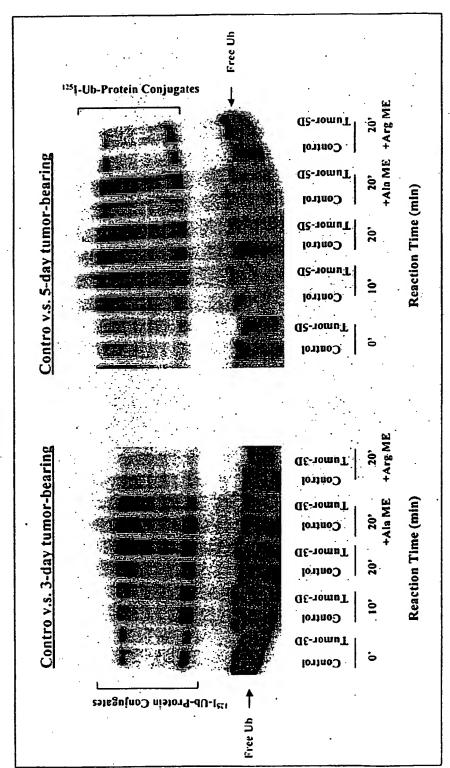




Figure 7

Rates of Ubiquitination of N-end Rule Substrate α-Lactalbumin in Skeletal Muscle Extracts

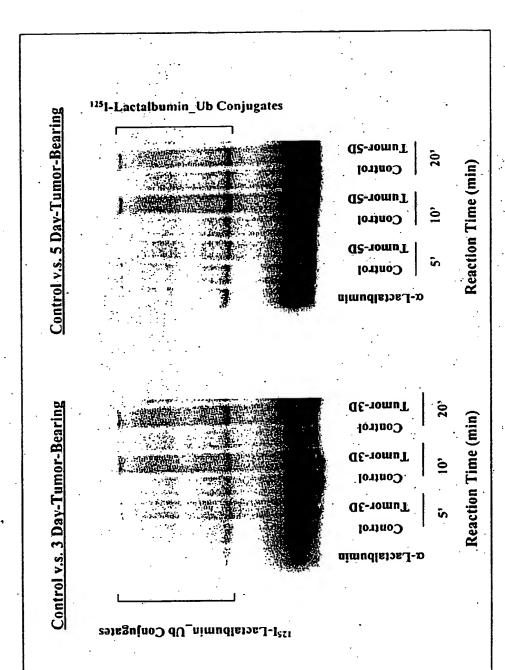




Figure 8

lvsis of E3\alpha-I & E3\alpha-II expression

ia mode	ջ ը Կօաղ		
exprimental caches	Sontrol, d3 Eg Sb, fortrol		
Northern blot analysis of ESG-1 & ESG-11 expression in gastrochemius muscles in YAH-130 exprimental cachexia mode	inmor, d3		

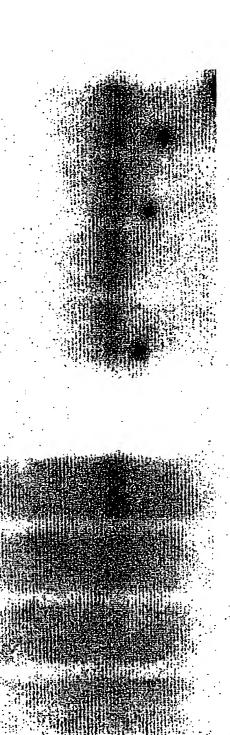


Figure 9

Northern blot analysis of E3 α -I and E3 α -II expression in gastrocnemius muscle and cardiac muscle in C26 experimental cachexia model

•		Gastroenmius	Heart
711	controj, c		
717	,bar inaq		
E302-1	tumor, d		
	control, c		
LIP	,bəî inaq		
. LI	tumor, d		
71p	control,		***
, d12	pari fed		
8 711 2 211	tomut.		
	control,		
/1p 'l	bəl insq		
<i>L</i> 1p	tumor,		



Figure 10

induce E3α-II Expression in C2C12 myostube culture Proinflammatory cytokines TNF- α and IL-6

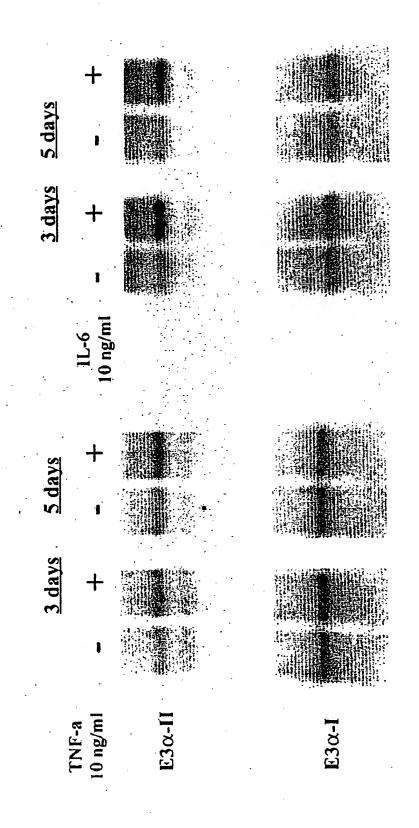
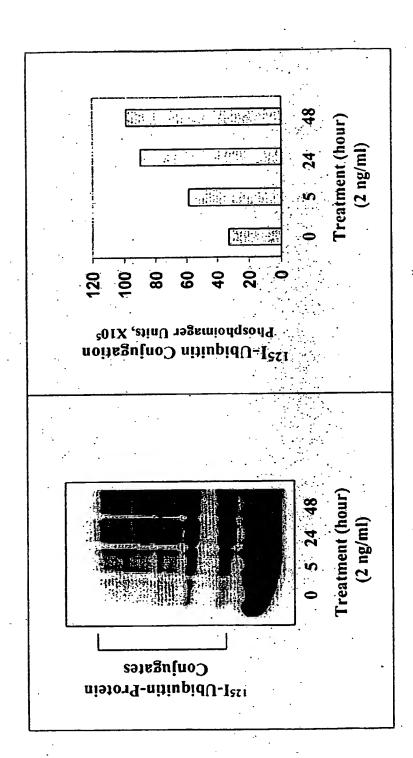




Figure 11

IL-6 Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures



PE STEE

 ${\sf TNF}_{\alpha}$ Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures Figure 12

